SYNOPSIS

1. TITLE OF THE THESIS

"PHYSICOCHEMICAL PROPERTIES AND KINETIC STUDIES ON NUCLEOPHILIC AND REACTIVATION POTENCIES OF SOME ACETYLCHOLINESTERASE REACTIVATORS"

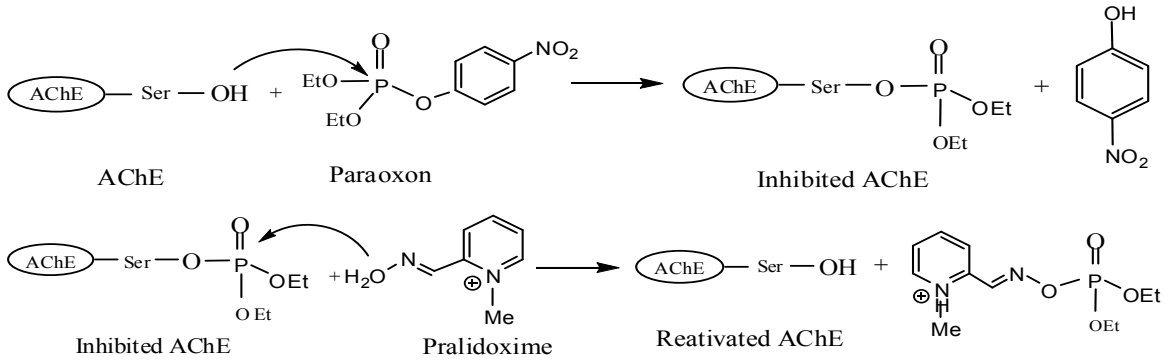
2. INTRODUCTION

Acetylcholinesterase (AChE) is a very important enzyme which hydrolyses acetylcholine and controls synaptic transmission of central and peripheral cholinergic synapses. [1-2]. This enzyme is a common target for toxic organophosphates like pesticides and nerve agents. These neurotoxic compounds can irreversibly inhibit the enzymatic activity. Inhibition of AChE occurs as a result of phosphylation of the active serine residue with organophosphorus compounds (OP) and nerve agents [3-4]. Such AChE inhibition results in acetylcholine accumulation at cholinergic receptor sites, thereby excessively stimulating the cholinergic receptors. This can lead to various clinical disorders, sometimes causing death. Reactivation of organophosphate inhibited AChE is very essential to get back its catalytic activity towards the hydrolysis of acetylcholine [5-6]. Till date there is no broad spectrum reactivator available for the treatment of organophosphorus poisoning. Therefore design, evaluation, kinetics and structure activity relationship studies of AChE reactivators against organophosphorus compounds are highly required. Most of the AChE reactivators are oximes and commonly used as α-nucleophilic reactivators of phosphorylated and phosphonylated AChE.

In the present investigation an attempt will be made to study physicochemical properties and kinetic studies of nucleophilic and reactivation potencies of some novel oxime based reactivators. Study of structure-activity relationship helps to design novel oxime reactivators. Quaternary pyridinium oximes (mono-,bis-) such as 2-hydroxyiminomethyl-1-methylpyridinium chloride (2-PAM) and several other oximes have been developed in the past as reactivators of OP poisoned AChE [7-8]. However

only few of them as 2-PAM, 1,3-bis-(4-hydroxyiminomethylpyridino-1-yl) propane dibromide (TMB-4) , 1-(2-hydroxyiminomethylpyridino-1-yl)-3-(4-carbamoylpyridino-1-yl)-2-oxapropane dichloride (HI-6) and 1,3-bis-(4-hydroxyiminomethylpyridino-1-yl)-2-oxapropane dichloride (Obidoxime) are available as antidotes against OP poisoning [9-10].

Most of the available oximes can't penetrate blood brain barrier. According to basic pharmacokinetic principles the blood brain barrier (BBB) is relatively impermeable to hydrophilic drugs like quaternary oximes due to quaternary structures and hydrophilic oxime groups, hence octanol-water partition coefficient [log *P*], which is based on the concept of partition of substances between oil and water, is a useful parameter for the prediction of drug transport properties and is also important for evaluating the ability of different drugs to penetrate through the BBB [11-12]. The pK_a values should also be taken into consideration while searching for new oxime reactivators essentially due to the fact that deprotonated oxime group is involved in the nucleophilic attack during reactivation and its concentration is pH dependant, therefore for the calculation of effective reactivation parameters, the effective concentration of oxime at particular pH should be determined [13-14]. Most of the oximes are much effective against inhibited AChE, so in order to diagnose the structural requirements of oxime it is necessary to study their reactivation kinetics. Kinetic approach leads to the assessment of oxime efficacy and mechanism of oxime action too. Understanding the mechanism of reactivation will help to design some better nerve agent antidotes. Scheme-I shows general mechanism of inhibition (paraoxon) and reactivation (2-PAM) of AChE.



Scheme I: Inhibition of AChE by paraoxon and reactivation by pralidoxime.

In the present investigation physicochemical properties and kinetics of nucleophilic potencies of some oxime based AChE reactivators will be studied. Reactivation kinetics of inhibited AChE in the presence of different oximes will be monitored. Effect of some suitable nanoparticles on the rate of reactivation will also be considered.

3. REVIEW OF THE EARLIER WORK

During the past decades, numerous studies have investigated the mechanism of oxime reactivation of OP-inhibited AChE. The search for oxime based reactivators led to the discovery of efficient AChE reactivators including some mono- and bis quaternary pyridinium oximes [15]. These reactivators are widely studied for the reactivation of OP inhibited AChE. The significant contribution of Kuca et al. [16] and Acharya et al. [13] deserves special mention in this context. In addition to these Taylor, Worek and other groups made special contributions. Taylor and coworkers [17] have investigated the new structural scaffolds of centrally acting oxime reactivators for phosphylated AChE. Significant contributions in this field are made by Worek et al. [18] as they studied kinetic analysis of interactions between human OP inhibited AChE and oximes. Nanoparticulate transport of oximes over an in vitro blood brain barrier model has been studied by Briesen et al [19]. Skivora and research group [20] have documented the use of tertiary oximes for the reactivation of intoxicated AChE.

Recently Churchill et al. [21] have published a review article on destruction and detection of chemical warfare agents, along with the history of pesticides, molecular structures and their toxicology. In vitro reactivation of paraoxon inhibited electric eel and human AChE with bis-quaternary pyridinium oximes have been studied by Thompson et al [22]. Design, synthesis and evaluation of α-nucleophiles for the hydrolysis of organophosphorus nerve agents and their application to the reactivation of phosphorylated acetylcholinesterase have been documented by Renard et al [23].

Inspite of tremendous efforts, a single broad spectrum AChE reactivator that may be used against all kinds of chemical warfare agents, is still lacking [24]. This fact underlines the necessity to develop an effective medical treatment for the whole range of

OP and nerve agents. Some preliminary studies on nucleophilic reactivity of oximes and hydroxamic acids have already been published from our laboratory.

4. NOTEWORTHY CONTRIBUTION IN THE FIELD OF PROPOSED WORK

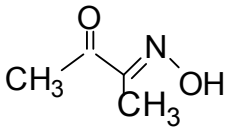
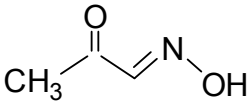
Organophosphorus compounds are among the most toxic compounds synthesized. They have been used as pesticides and nerve agents for several decades. The accumulation of these compounds in the environment is a recognized ecological threat existing in the global scenario. Many scientists are trying to search novel antidotes based on physicochemical parameters of various AChE reactivators. In addition to that many novel oxime based acetylcholinesterase reactivators have been developed. The efficiency of the reactivator is attributed to the nucleophilicity of the oximate ions and the decay rate of the intermediate is dependent on the structure of the oxime. In order to get insight into the structural requirements of oximes to reactivate OP inhibited AChE, it is indispensible to study the physicochemical properties and reactivation rate constants of variety of AChE reactivators. In the last few years considerable efforts have been made by our group to study the structure, kinetics and properties of various oximate ions and their role in detoxification chemistry [25-29].

5. PROPOSED METHODOLOGY DURING THE TENURE OF THE RESEARCH WORK

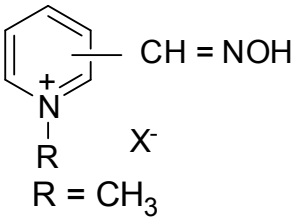
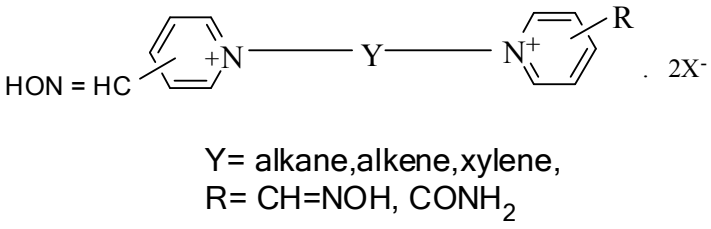
The entire experimental work will be carried out in the following steps:

I. Synthesis of AChE Reactivators

The synthesis and characterization of some AChE reactivators will be done in collaboration with Dr. Kamil Kuca (Center of Advanced Studies and Department of toxicology, Faculty of Military Health Sciences, Department of Chemistry Czech Republic) and Dr. J. Acharya (Defence Research and Development Establishment, Gwalior). Some commercially available tertiary oximes (Scheme II) will also be used. The general structures of some novel oxime based reactivators are shown in scheme III.

	
butane 2, 3-dione monoxime (BDMO)	Monoisonitroso acetone (MINA)

Scheme II: Structures of some commercially available tertiary oxime.

	
mono-pyridinium oxime	bis-pyridinium oxime

III. General structure of mono and bis-pyridinium oxime reactivators

II Physicochemical properties of some mono- and bis-quaternary pyridinium oximes.

The partition coefficient (log *P*) of some novel synthesized pyridinium oximes will be studied spectrophometrically and comparisons will be made with commercially available oximes and hydroxamic acids. The acid dissociation constants (pK_a) of these synthesized reactivators will also be studied using spectrophotometric and kinetic methods.

III Kinetics of nucleophilic potencies of oximate ions towards hydrolysis of carboxlyate, phosphate and sulphonate esters in micellar media.

Kinetic studies of the hydrolysis of different phosphate, thiophosphate and sulphonate esters will be studied in the presence of various oximate and hydroxamate α-nucleophiles in the presence and absence of micellar media. Their nucleophilic potencies will also be compared.

IV Study of reactivation kinetics of OP inhibited AChE.

Efficacy of oxime reactivators against OP inhibited electric eel AChE will be studied using Ellman's method. Organophosphorus compounds viz. Paraoxon, Parathion, Fenitrothion and other toxic and non-toxic phosphate esters will be used as AChE inhibitors. Enzyme reactivation kinetics will be monitored against time and concentration of reactivators.

V Use of nanoparticles.

It has been surveyed that the nanoparticles enable a better transport of drug over the BBB than free or only oxime moiety, therefore an attempt will be made to study the oxime reactivators in the presence of some suitable nano particles. *In-vitro* reactivation studies will be performed in the presence of some synthesized nanoparticles.

6. EXPECTED OUTCOME

The chemical transformation of toxic organophosphorus compounds is highly relevant, as there is continuing threat of the usage of chemical warfare agents. The development of novel oxime based compounds and their role in reactivating enzyme AChE will be an important area of defense and academic research. Owing to the threat of organophosphate exposures, not only to pesticides but also nerve agents, it is very important to know the whole process of organophosphates-inhibited AChE reactivation. Although current antidotes against organophosphorus compounds are available, AChE reactivators are still needed, especially in the case of intoxications with high doses of organophosphates. Hence the results of present investigation regarding structure-activity analysis and kinetics of AChE reactivators will be of great significance. The results of this research will enrich our understanding about reactivators of AChE and their action and promising role in detoxification purposes.

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1. International Symposium on Novel approaches to air quality management and improvement in India, 5-7 Sep. 2011. (**Attended**)
Organized by- SoS in chemistry, Pt. R.S.S.U. Raipur.

2. National Seminar on Recent Trends in Chemical Sciences and Future Prospects, 11-12 Oct. 2011. (**Attended**).
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3. "Structural and Kinetic Aspects of Pyridinium Oximes as Reactivators of Organophosphate inhibited Acetylcholinesterase"
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